

**§ 610.12 Sterility.**

Except as provided in paragraphs (f) and (g) of this section, the sterility of each lot of each product shall be demonstrated by the performance of the tests prescribed in paragraphs (a) and (b) of this section for both bulk and final container material.

(a) *The test.* Bulk material shall be tested separately from final container material and material from each final container shall be tested in individual test vessels as follows:

(1) *Using Fluid Thioglycollate Medium—(i) Bulk and final container material.* The volume of product, as required by paragraph (d) of this section (hereinafter referred to also as the “inoculum”), from samples of both bulk and final container material, shall be inoculated into test vessels of Fluid Thioglycollate Medium. The inoculum and medium shall be mixed thoroughly and incubated at a temperature of 30 to 35 °C for a test period of no less than 14 days and examined visually for evidence of growth on the third, fourth, or fifth day, and on the seventh or eighth day, and on the last day of the test period. Results of each examination shall be recorded. If the inoculum renders the medium turbid so that the absence of growth cannot be determined reliably by visual examination, portions of this turbid medium in amounts of no less than 1.0 milliliter shall be transferred on the third, fourth, or fifth day of incubation, from each of the test vessels and inoculated into additional vessels of the medium. The material in the additional vessels shall be incubated at a temperature of 30 to 35 °C for no less than 14 days. Notwithstanding such transfer of material, examination of the original vessels shall be continued as prescribed above. The additional test vessels shall be examined visually for evidence of growth on the third, fourth, or fifth day of incubation, and on the seventh or eighth day, and on the last day of the incubation period. If growth appears, repeat tests may be performed as prescribed in paragraph (b) of this section and interpreted as specified in paragraph (c) of this section.

(ii) *Final container material containing a mercurial preservative.* In addition to the test prescribed in paragraph

(a)(1)(i) of this section, final container material containing a mercurial preservative shall be tested using Fluid Thioglycollate Medium following the procedures prescribed in such subparagraph, except that the incubation shall be at a temperature of 20 to 25 °C.

(2) *Using Soybean-Casein Digest Medium.* Except for products containing a mercurial preservative, a test shall be made on final container material, following the procedures prescribed in paragraph (a)(1)(i) of this section, except that the medium shall be Soybean-Casein Digest Medium and the incubation shall be at a temperature of 20 to 25 °C.

(b) *Repeat tests.* If growth appears in any of the test media during testing of either bulk or final container material, the test may be repeated to rule out faulty test procedures as follows:

(1) *Repeat bulk test.* Only one repeat bulk test may be conducted. The volume of inoculum to be used for the repeat bulk test shall be as prescribed in paragraph (d)(1) of this section. The repeat test shall be performed using the procedure prescribed in paragraph (a)(1)(i) of this section.

(2) *First repeat final container test.* The number of test samples and the volumes of product used for the first repeat test shall be as prescribed in paragraph (d)(2) of this section. For products that do not contain a mercurial preservative, the repeat test shall be performed, using both Fluid Thioglycollate Medium and Soybean-Casein Digest Medium, following the procedures prescribed in paragraphs (a)(1)(i) and (a)(2), respectively, of this section. If the product contains a mercurial preservative, the repeat test shall be performed using Fluid Thioglycollate Medium and the procedures prescribed in paragraphs (a)(1) (i) and (ii) of this section.

(3) *Second repeat final container test.* If growth appears in any of the first repeat final container tests, all tests of the first repeat final container test shall be repeated, provided there was no evidence of growth in any test of the bulk material. The test samples used for the second repeat final container test shall be twice the number used for the first repeat final container test.

(c) *Interpretation of test results.* The results of all tests performed on a lot shall be considered in determining whether or not the lot meets the requirements for sterility, except that tests may be excluded when demonstrated by adequate controls to be invalid. The lot meets the test requirements if no growth appears in the tests prescribed in paragraph (a) of this section. If repeat tests are performed, the lot meets the test requirements if no growth appears in the tests prescribed in paragraph (b)(2) or (3) of this section, whichever is applicable.

(d) *Test samples and volumes*—(1) *Bulk.* Each sample for the bulk sterility test shall be representative of the bulk material and the volume tested shall be no less than 10 ml. (Note exceptions in paragraph (g) of this section.)

(2) *Final containers.* The sample used for each test medium or each incubation temperature of a test medium for the final container and first repeat final container test shall be no less than 20 final containers from each filling of each lot, selected to represent all stages of filling from the bulk vessel. If the amount of material in the final container is 1.0 milliliter or less, the entire contents shall be tested. If the amount of material in the final container is more than 1.0 milliliter, the volume tested shall be the largest single dose recommended by the manufacturer or 1.0 milliliter, whichever is larger, but no more than 10 milliliters of material or the entire contents from a single final container need be tested. If more than 2 filling machines, each with either single or multiple filling stations, are used for filling one lot, no less than 10 filled containers shall be tested from each filling machine for each test medium or each incubation temperature condition, but no more than 100 containers of each lot need be tested. The items tested shall be representative of each filling assembly and shall be selected to represent all stages of the filling operation. (Note exceptions in paragraph (g) of this section.)

(e) *Culture medium*—(1) *Formulae.* (i) The formula for Fluid Thioglycollate Medium is as follows:

#### FLUID THIOGLYCOLLATE MEDIUM

1-cystine .....	0.5 gm.
Sodium chloride .....	2.5 gm.
Dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ) .....	5.5 gm.
Granular agar (less than 15% moisture by weight) .....	0.75 gm.
Yeast extract (water-soluble) .....	5.0 gm.
Pancreatic digest of casein .....	15.0 gm.
Purified water .....	1,000.0 ml.
Sodium thioglycollate (or thioglycolic acid—0.3 ml) .....	0.5 gm.
Resazurin (0.10% solution, 1.0 ml. freshly prepared) .....	
pH after sterilization .....	7.1±0.2.

(ii) The formula for Soybean-Casein Digest Medium is as follows:

#### SOYBEAN-CASEIN DIGEST MEDIUM

Pancreatic Digest of Casein .....	17.0 gm.
Papaic Digest of Soybean Meal .....	3.0 gm.
Sodium Chloride .....	5.0 gm.
Dibasic Potassium Phosphate .....	2.5 gm.
Dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ) .....	2.5 gm.
Purified water .....	1,000.0 ml.
pH after sterilization .....	7.3±0.2.

(2) *Culture media requirements*—(i) *Definition of a lot of culture medium and test requirements.* A lot of culture medium is that quantity of uniform material identified as having been thoroughly mixed in a single vessel, dispensed into a group of vessels of the same composition and design, sterilized in a single autoclave run, and identified in a manner to distinguish one lot from another. Each lot of culture medium shall be tested for its growth-promoting qualities unless it meets the exception for dehydrated culture medium described in this subpart. The growth-promoting quality test shall be performed on the smallest sized vessel used in an autoclave run. When using a single batch of dehydrated culture medium, a manufacturer need not perform growth-promoting tests on each lot of prepared liquid medium, provided that a validation program exists for autoclaves used to sterilize the culture medium, and the manufacturer has received approval for this practice from the Director, Center for Biologics Evaluation and Research or the Director, Center for Drug Evaluation and Research.

(ii) *Test organisms, strains, characteristics, identity, and verification.* Two or more strains of microorganisms that are exacting in their nutritive and aerobic/anaerobic requirements shall be used to test the growth-promoting qualities of each lot of test medium.

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When using Fluid Thioglycollate medium, both an aerobic and an anaerobic test microorganism shall be chosen. When using Soybean Casein Digest Medium, the yeast, *Candida albicans*, shall

be one of the two test microorganisms chosen. Manufacturers shall choose the strains of microorganisms from the chart in this paragraph.

Medium	Test microorganisms	Incubation temperature
Fluid Thioglycollate .....	<i>Spore-formers</i>	
	1. <i>Bacillus subtilis</i> (ATCC No. 6633) .....	30 to 35 °C.
	2. <i>Clostridium sporogenes</i> (ATCC No. 11437) .....	Do.
	<i>Non-spore-formers</i>	
	3. <i>Candida albicans</i> (ATCC No. 10231) .....	Do.
	4. <i>Micrococcus luteus</i> (ATCC No. 9341) .....	Do.
Soybean-Casein Digest .....	5. <i>Bacteroides vulgatus</i> (ATCC No. 8482) .....	Do.
	<i>Spore-formers</i>	
	1. <i>Bacillus subtilis</i> (ATCC No. 6633) .....	20 to 25 °C.
	<i>Non-spore-formers</i>	
	2. <i>Candida albicans</i> (ATCC No. 10231) .....	Do.
	3. <i>Micrococcus luteus</i> (ATCC No. 9341) .....	Do.

ATCC strains of microorganisms described in this section are available from the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110. Periodic tests shall be performed to verify the integrity of the test organisms in accordance with §610.18 (a) and (b). The results of these periodic tests shall be recorded and retained in accordance with §600.12(b) of this chapter.

(iii) *Storage and maintenance of cultures of test organisms.* Cultures of the test organisms used to determine the growth-promoting qualities of the medium shall be stored in a manner that will prevent cross contamination or loss of identity, at a temperature and by a method that will retain the initial characteristics of the organisms and ensure freedom from contamination and deterioration. If the test organisms are stored in the freeze-dried state, or frozen, they shall be reconstituted or thawed, whichever is applicable, and plated periodically to verify the colony count of the suspension. If the test suspensions are stored in a state other than freeze-dried or frozen, they shall be plated, and a colony count shall be performed at the time of each growth-promoting quality test to assure that not more than 100 organisms are used per test vessel. The results of tests for verification of the colony count shall be recorded and retained in accordance with §600.12(b) of this chapter.

(iv) *Storage and condition of media.* A medium shall not be used if the extent of evaporation affects its fluidity, nor shall it be reused in a sterility test of the product. Fluid Thioglycollate Medium shall be stored in the dark at room temperature if the vessels are unsealed. Sealed vessels shall be stored at the manufacturer's specified storage temperature. Fluid Thioglycollate Medium shall not be used if more than the upper one-third of the medium has acquired a pink color. The medium may be restored once by heating on a steam bath or in free-flowing steam until the pink color disappears. The design of the test vessel for Fluid Thioglycollate Medium shall provide favorable aerobic and anaerobic conditions for growth of the microorganisms throughout the test period. Soybean-Casein Digest Medium shall be stored in the dark at 20 to 25 °C. Unsealed vessels of either medium may be stored for more than 10 days at the proper temperature, provided they are tested monthly for growth-promotion and found to be satisfactory. Sealed vessels of either medium may be stored at the proper temperature for a period of time not to exceed 1 year, provided they are tested for growth-promotion every 3 months and found to be satisfactory. The results of such testing shall be recorded and retained in accordance with §600.12(b) of this chapter.

(v) *Criteria for a satisfactory growth-promoting quality test.* (a) One hundred or fewer organisms of each strain tested shall be used. The test is satisfactory if evidence of growth appears within 7 days in all vessels inoculated. If a lot of medium fails to support the growth of any test organism, or if the test results show that more than 100 organisms of a strain were used or are necessary to promote growth in the lot of medium being tested, or if the growth is not a pure culture of the test organism, a second test may be performed. If it fails the second test, the lot of medium shall be rejected.

(b) Inoculated Fluid Thioglycollate Medium shall be incubated at 30 to 35 °C for 7 days. If the test medium is to be used in determining the sterility of a product containing a mercurial preservative, a second test shall be performed in accordance with paragraph (e)(2)(v)(a) of this section, except that the test shall be incubated at 20 to 25 °C for 7 days. Inoculated Soybean-Casein Digest Medium shall be incubated at 20 to 25 °C for 7 days. The sterility of each lot of medium shall be confirmed by the incubation of uninoculated control test vessels for 7 days at the temperature(s) for that particular medium. The lot of medium is satisfactory if no growth is observed in the control test vessels within the incubation period. The tests for growth-promoting qualities of culture media may be performed simultaneously with sterility testing of biological products, provided the sterility test is considered invalid if the test medium shows no growth response.

(vi) *Volume of culture medium.* The volume of each culture medium shall be determined for each bulk and final container sterility test required for each product. The ratio of the volume of inoculum to the volume of culture medium shall result in a dilution of the product that is not bacteriostatic or fungistatic, except for products to be tested by membrane filtration. The volume of inhibitors or neutralizers of preservatives added should be considered in determining the proper ratio of inoculum/medium. Vessels of the product-medium mixture(s) and control vessels of the medium shall be inoculated with dilutions of cultures of bac-

teria or fungi which are viable in the product being tested, and incubated at the appropriate temperature for no less than 7 days.

(f) *Membrane filtration.* Bulk and final container material or products containing oil products in water-insoluble ointments may be tested for sterility using the membrane filtration procedure set forth in the United States Pharmacopeia (23d Revision, 1995), section entitled "Test Procedures Using Membrane Filtration," pp. 1689 to 1690, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the United States Pharmacopeial Convention, Inc., 12601 Twinbrook Pkwy., Rockville, MD 20852, or available for inspection at the Center for Drug Evaluation and Research's Division of Medical Library, 5600 Fishers Lane, rm. 11B-40, Rockville, MD, or at the National Archives and Records Administration (NARA). (For information on the availability of this material at NARA, call 202-741-6030, or go to: [http://www.archives.gov/federal\\_register/code\\_of\\_federal\\_regulations/ibr\\_locations.html](http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html)). Except that:

(1) The test samples shall conform with paragraph (d) of this section; and

(2) In addition, for products containing a mercurial preservative, the product shall be tested in a second test using Fluid Thioglycollate Medium incubated at 20 to 25½°C in lieu of the test in Soybean-Casein Digest Medium.

(g) *Exceptions.* Bulk and final container material shall be tested for sterility as described above in this section, except as follows:

(1) *Different sterility tests prescribed.* When different sterility tests are prescribed for a product in this subchapter.

(2) *Alternate incubation temperatures.* Two tests may be performed as prescribed in paragraph (a)(1)(i) of this section, one test using an incubation temperature of 18 to 22 °C, the other test using an incubation temperature of 30 to 37 °C, in lieu of performing one test using an incubation temperature of 30 to 35 °C, provided that growth-promoting quality tests have been performed at these temperatures.

(3) [Reserved]

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(4) *Test precluded or not required.* (i) The tests prescribed in this section need not be performed for Whole Blood, Cryoprecipitated AHF, Platelets, Red Blood Cells, Plasma, Source Plasma, Smallpox Vaccine, Reagent Red Blood Cells, Anti-Human Globulin, or Blood Grouping Reagent.

(ii) Where a manufacturer submits data which the Director, Center for Biologics Evaluation and Research or the Director, Center for Drug Evaluation and Research, finds adequate to establish that the mode of administration, the method of preparation, or the special nature of the product precludes or does not require a sterility test or that the sterility of the lot is not necessary to assure the safety, purity, and potency of the product, the Director may exempt a product from the sterility requirements of this section subject to any conditions necessary to assure the safety, purity, and potency of the product.

(5) *Number of final containers more than 20, less than 200.* If the number of final containers in the filling is more than 20 or less than 200, the sample shall be no less than 10 percent of the containers.

(6) *Number of final containers—20 or less.* If the number of final containers in a filling is 20 or less, the sample shall be two final containers, or the sample need be no more than one final container, provided (i) the bulk material met the sterility test requirements and (ii) after filling, it is demonstrated by testing a simulated sample that all surfaces to which the product was exposed were free of contaminating microorganisms. The simulated sample shall be prepared by rinsing the filling equipment with sterile 1.0 percent peptone solution, pH 7.1  $\pm$  0.1, which shall be discharged into a final container by the same method used for filling the final containers with the product.

(7) *Samples—large volume of product in final containers.* For Albumin (Human) and Plasma Protein Fraction (Human), when the volume of product in the final container is 50 milliliters or more, the final containers selected as the test sample may contain less than the full volume of product in the final containers of the filling from which the sample is taken: *Provided*, That the

containers and closures of the sample are identical with those used for the filling to which the test applies, and the sample represents all stages of that filling.

(8) *Diagnostic biological products not intended for injection.* For diagnostic biological products not intended for injection, (i) only the Fluid Thioglycollate Medium test incubated at 30 to 35 °C is required, (ii) the volume of material for the bulk test shall be no less than 2.0 milliliters, and (iii) the sample for the final container test shall be no less than three final containers if the total number filled is 100 or less, and, if greater, one additional container for each additional 50 containers or fraction thereof, but the sample need be no more than 10 containers.

(9) *Immune globulin preparations.* For immune globulin preparations, the test samples from the bulk material and from each final container need be no more than 2.0 ml.

(h) *Records.* The records related to the testing requirements of this section shall be prepared and maintained as required by §§211.167 and 211.194 of this chapter.

[38 FR 32056, Nov. 20, 1973, as amended at 41 FR 4015, Jan. 28, 1976; 41 FR 10428, Mar. 11, 1976; 44 FR 11754, Mar. 2, 1979; 49 FR 15187, Apr. 18, 1984; 49 FR 23834, June 8, 1984; 50 FR 4133, Jan. 29, 1985; 51 FR 44906, Dec. 15, 1986; 53 FR 12764, Apr. 19, 1988; 55 FR 11013, Mar. 26, 1990; 62 FR 48175, Sept. 15, 1997; 67 FR 9587, Mar. 4, 2002; 69 FR 18803, Apr. 9, 2004; 70 FR 14985, Mar. 24, 2005]

### §610.13 Purity.

Products shall be free of extraneous material except that which is unavoidable in the manufacturing process described in the approved biologics license application. In addition, products shall be tested as provided in paragraphs (a) and (b) of this section.

(a)(1) *Test for residual moisture.* Each lot of dried product shall be tested for residual moisture and shall meet and not exceed established limits as specified by an approved method on file in the biologics license application. The test for residual moisture may be exempted by the Director, Center for Biologics Evaluation and Research or the Director, Center for Drug Evaluation